Anti-Neurofilament heavy polypeptide Antibody [JF99-07]

ET1702-72

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IHC-Fr, IF-Tissue
Molecular Wt:	Predicted band size: 200 kDa
Clone number:	JF99-07

Description: Neurofilament-H (NF-H), for neurofilament heavy polypeptide, a member of the intermediate filament family, is a major component of neuronal cytoskeletons. Neurofilaments are dynamic structures; they contain phosphorylation sites for a large number of protein kinases, including protein kinase A, protein kinase C, cyclin-dependent kinase 5, extracellular signal regulated kinase, glycogen synthase kinase-3, and stress-activated protein kinase gamma. In addition to their role in the control of axon caliber, neurofilaments may affect other cytoskeletal elements, such as microtubules and Actin filaments. Changes in neurofilament phosphorylation or metabolism are frequently observed in neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), Parkinson's disease and Alzheimer's disease.

Immunogen: Synthetic peptide within human NEFH aa 690-740.

Positive control: Mouse brain tissue lysate, Rat brain tissue lysate, Rat hippocampus tissue lysate, human cerebellum tissue, mouse cerebellum tissue, rat cerebellum tissue.

Subcellular location: Cytoplasm.

Database links: SwissProt: P12036 Human | P19246 Mouse | P16884 Rat

Recommended Dilutions:

WB	1:2,000
IHC-P	1:1,000
IHC-Fr	1:500
IF-Tissue	1:500

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

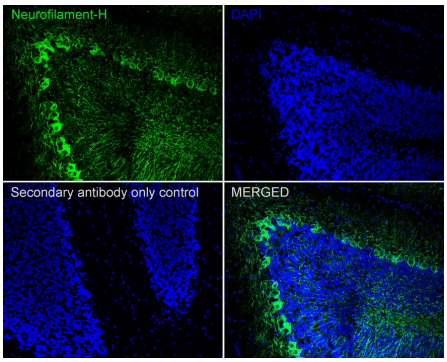

Fig1: Immunofluorescence analysis of frozen mouse cerebellum tissue with Rabbit anti-Neurofilament heavy polypeptide antibody (ET1702-72) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1702-72, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

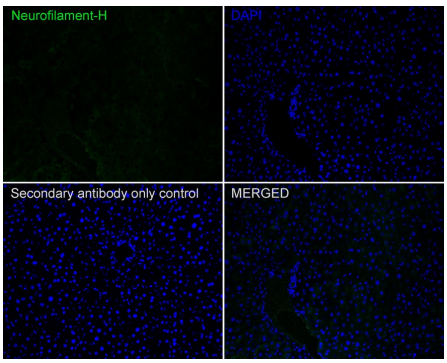

Fig2: Immunofluorescence analysis of frozen mouse liver tissue (negative) with Rabbit anti-Neurofilament heavy polypeptide antibody (ET1702-72) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1702-72, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

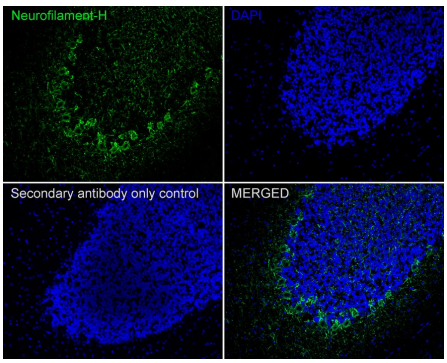

Fig3: Immunofluorescence analysis of frozen rat cerebellum tissue with Rabbit anti-Neurofilament heavy polypeptide antibody (ET1702-72) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1702-72, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

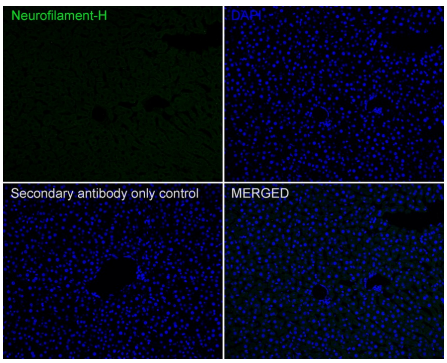

Fig4: Immunofluorescence analysis of frozen rat liver tissue (negative) with Rabbit anti-Neurofilament heavy polypeptide antibody (ET1702-72) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1702-72, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

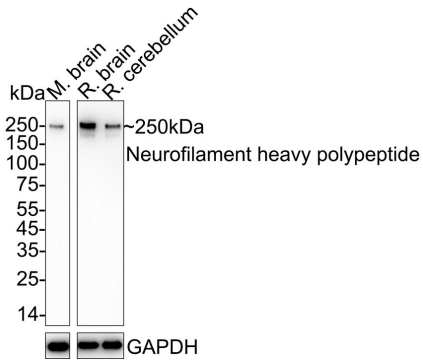

Fig5: Western blot analysis of Neurofilament heavy polypeptide on different lysates with Rabbit anti-Neurofilament heavy polypeptide antibody (ET1702-72) at 1/2,000 dilution.

Lane 1: Mouse brain tissue lysate (20 µg/Lane)
Lane 2: Rat brain tissue lysate (20 µg/Lane)
Lane 3: Rat hippocampus tissue lysate (20 µg/Lane)

Predicted band size: 200 kDa
Observed band size: 250 kDa

Exposure time: 3 minutes; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1702-72) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

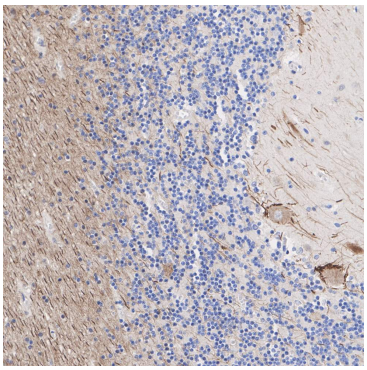

Fig6: Immunohistochemical analysis of paraffin-embedded human cerebellum tissue with Rabbit anti-Neurofilament heavy polypeptide antibody (ET1702-72) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1702-72) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

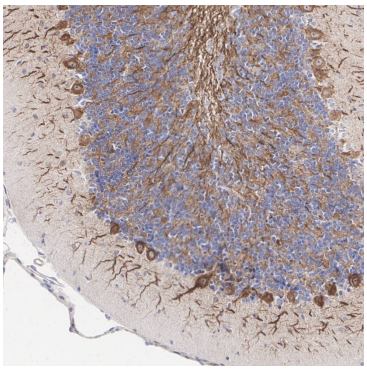

Fig7: Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue with Rabbit anti-Neurofilament heavy polypeptide antibody (ET1702-72) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1702-72) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

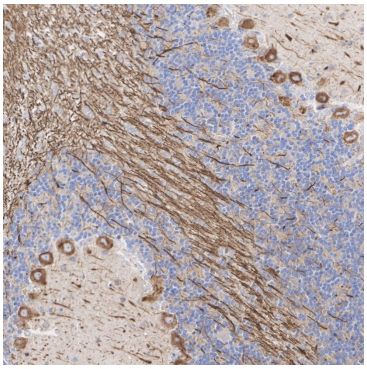

Fig8: Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue with Rabbit anti-Neurofilament heavy polypeptide antibody (ET1702-72) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1702-72) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

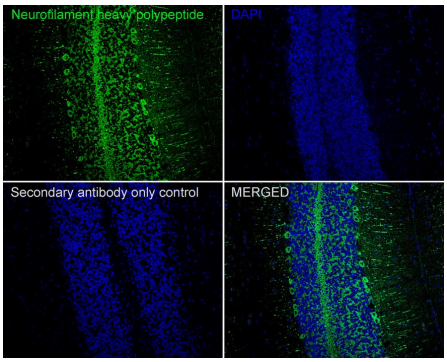

Fig9: Immunofluorescence analysis of paraffin-embedded mouse cerebellum tissue labeling Neurofilament heavy polypeptide with Rabbit anti-Neurofilament heavy polypeptide antibody (ET1702-72) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1702-72, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

Background References

1. Slowik A et al. The sphingosine 1-phosphate receptor agonist FTY720 is neuroprotective after cuprizone-induced CNS demyelination. Br J Pharmacol 172:80-92 (2015).
2. Patra K et al. A role for solute carrier family 10 member 4, or vesicular aminergic-associated transporter, in structural remodelling and transmitter release at the mouse neuromuscular junction. Eur J Neurosci 41:316-27 (2015).